PATENT IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

IN RE APPLICATION OF: NAGAIKE *et al.*	EXAMINER: CHEN, Stacy Brown
APPLICATION NO.: 10/591,787	ART UNIT: 1648
371(C) DATE: July 30, 2007	CONF. NO: 3253
FOR: **RECOMBINANT VARICELLA-ZOSTER VIRUS**	

DECLARATION UNDER 37 C.F.R. § 1.132

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Sir:

I, KAZUHIRO NAGAIKE, declare and affirm as follows:

1. I am currently employed as the Section Manager in The Research Foundation for Microbial Diseases of Osaka University, having held such position since April, 2011. I have worked with this company since April 1993 in various positions.

2. I received a Doctor of Philosophy degree in Medical Science at the University of Osaka in 2008.

3. I am a named inventor on U.S. Patent Application 10/591,787, and I supervised, or participated in, and/or have firsthand knowledge of the studies reported below.

Background: Comparison of DNA sequences of the Oka parent and vaccine strains:

4. As is noted in Exhibit A (Gomi, *et al.*, J Virology, 2002, p. 11447-11459, previously made of record in the Information Disclosure Statement (IDS) mailed 11 December 2006, and re-submitted herewith for the Examiner's convenience), we have performed experiments to determine specific gene regions of the Varicella Zoster Virus (VZV) genome which are not essential for expression. When gene 13 of was disrupted by inserting a kanamycin resistance gene, the virus proliferated and produced virus particles, thus identifying VZV13 as non-essential. 42 base substitutions (leading to 20 amino acid substitutions) have been found in a comparison of the complete DNA sequences of the Oka Varicella parent strain (also known as "wild type") and the Oka Varicella vaccine strain (also known as "mutant"). Table I, below, is

based on Table 3 in the Gomi reference, and illustrates these differences between Oka parent strain and Oka vaccine strain, and compares two additional strains isolated from Oka vaccine strain (S7-01 and S7-13).

Table I

position	gene	Oka parent	Oka vaccine	S7-01	S7-13	function
5745 nt	gene 6	A (Gln)	G (Pro)	G (Pro)	G (Pro)	helicase/primase complex
10900	gene 9A	T (Trp)	T/C (Trp/Arg)	T (Trp)	C (Arg)	unknown
12779	gene 10	T (Ala)	T/C (Ala/Val)	C (Val)	C (Val)	α-TIF
31732	gene 21	C (Thr)	C/T (Thr/Ile)	T (Ile)	C (Thr)	latent associated protein
58695	gene 31	A (Ile)	A/G (Ile/Val)	G (Val)	A (Ile)	gB
71252	gene 39	T (Met)	T/C (Met/Thr)	C (Thr)	C (Thr)	unknown
87306	gene 50	T (Ser)	T/C (Ser/Gly)	T (Ser)	T (Ser)	HSV-1 gM homolog
90535	gene 52	A (Ile)	A/G (Ile/Val)	A (Ile)	G (Val)	helicase/primase complex
97748	gene 55	G (Ala)	G/A (Ala/Thr)	G (Ala)	A (Thr)	helicase/primase complex
97796		T (Cys)	T/C (Cys/Arg)	C (Arg)	T (Cys)	
101089	gene 59	A (Leu)	A/G (Leu/Pro)	A (Leu)	A (Leu)	uracil-DNA glycosylase
105310	gene 62	A (Leu)	A/G (Leu/Ser)	G (Ser)	G (Ser)	IE62, transactivator
105356		T (Val)	C (Val)	C (Val)	C (Val)	
105544		A (Ala)	G (Ala)	G (Ala)	G (Ala)	
106262		A (Arg)	C (Gly)	C (Gly)	C (Gly)	
107252		T (Gly)	C (Gly)	C (Gly)	C (Gly)	
107599		A (Val)	A/G (Val/Ala)	G (Ala)	A (Val)	
107797		A (Leu)	A/G (Leu/Pro)	G (Pro)	A (Leu)	
108838		A (Met)	A/G (Met/Thr)	G (Thr)	A (Met)	
111650	gene 64	A (Gln)	A/G (Gln/Arg)	G (Arg)	A (Gln)	unknown

As can be seen in the table, the 42 base substitutions (20 amino acid substitutions) were found in 12 genes (genes 6, 9A, 10, 21, 31, 39, 50, 52, 55, 59, 62, and 64). Among these substitutions, 15 base substitutions (8 amino acid substitutions) were concentrated in gene 62. Because the strains having a greater number of amino acid substitutions in gene 62 showed smaller plaque formation and less efficient virus spreading activity than the parental strain, gene 62 of VZV appears to be involved in attenuation of the vaccine strain.

Differences between HSV and VZV

5. There are clear structural and functional differences between Herpes Simplex Virus (HSV) described in Horsburgh *et al.* (US Patent 6,277,621) and VZV. First, VZV comprises about 70 genes in a DNA genome of about 125 kbp, quite distinct from HSV, which has about 80 genes in a DNA genome of 150 kbp. The viruses are not directly comparable such that one can assume that a HSV gene # and a VZV gene # are homologous. Table II, below, illustrates

this fact. Note that VZV gene 13 has no counterpart in HSV, and UL13 cited by the Examiner is VZV47, a protein kinase Tegument protein. Our group has confirmed that when gene 13 was disrupted by inserting the kanamycin resistance gene, the virus proliferated and produced virus particles, thereby identifying gene 13 as non-essential.

Table II[1]

VZV gene	VZVgene function	Non-essential /Essential	HSV gene	Function of polypeptide encoded by the gene
7			UL51	
13	Thymidylate synthetase	Non-essential	No counterpart***	
21	Nucleocapsid protein	*Essential	UL37	
46			UL14	
48			UL12	deoxyribonucleease
56			UL4	
58			UL3	
66	Putative protein kinase	Non-essential	US3	
3			UL55	
8			UL50	
14	g C	Non-essential	UL44	
15			UL43	
17			UL41	virion shotoff protein
18	Ribonucleotide reductase, small subunit		UL40	
35			UL24	
36	Thymidine kinase	Non-essential	UL23	
39			UL20	
44			UL16	
47	Protein kinase, Tegument protein	Non-essential	UL13	
49			UL11	
50			UL10 (g M)	
56			UL4	
58	Uracil-DNA glycosylase	Non-essential	UL3	
63	Tegument protein		US1(IE68)	
64			US2	
66	Putative protein kinase	Non-essential	US3	The protein kinase gene
66	Putative protein kinase	Non-essential	US4	The glycoprotein G gene
66	Putative protein kinase	Non-essential	US5	
67	gI	Non-essential	US7	The glycoprotein I gene
68	gE		US8	The glycoprotein E gene
68	gE		US9	
68	gE		US10	

[1] *Gene 21 and gene 62(71) were identified as being essential gene after filing date (Blue colored box)
**there is no counterpart gene in VZV corresponding to genes UL45, UL46, UL47, and UL56.
***there exists no corresponding gene relating to VZV gene 13 of the present invention.

68	gE		US11	
68	gE		US12	IE12
6			UL52	Helicase-primase complex
62(71)	Transactivator, tegument protein	*Essential	ICP4(Vmw175)	

6. Furthermore, because HSV has strong neurotropism and high tumoricidal effects, HSV is employed in the medical field for gene therapy and viral delivery of various treatments, but the HSV vector of Horsburgh *et al.* would not be effective as a vaccine. The use of HSV requires acyclovir as a safe guard against uncontrolled viral proliferation, as acyclovir kills the virus via expression of the herpesvirus thymidine kinase (TK) gene. However, the TK gene is disrupted in the HSV vector produced according to Horsburgh *et al.*, and thus, the HSV vector of Horsburgh *et al.* could not be used as a vaccine. In contrast, the VZV Oka vaccine strain of the present invention is an attenuated recombinant live vaccine developed as a vaccine, where safety in its utility is guaranteed without the use of acyclovir.

7. In light of the apparent structural and functional differences between VZV non-essential genes of the claimed invention and the disclosure in *Horsburgh et al.* of non-essential genes of HSV, *Horsburgh et al.* is neither applicable nor relevant to the claimed invention.

8. I declare that all statements made herein of my own knowledge are true, and that all statements made on information or belief are believed to be true; and further, that these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of any patent issuing from this patent application.

Respectfully submitted,

May 19, 2011
Date

Kazuhiro Nagaike
KAZUHIRO NAGAIKE